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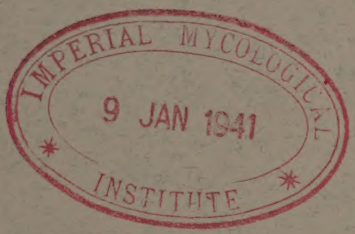
BY

W. BUDDIN AND E. M. WAKEFIELD



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STUDIES ON RHIZOCTONIA CROCORUM (PERS.) DC. AND HELICOBASIDIUM PURPUREUM (TUL.) PAT.*

(With Plates XI–XIV.)

By W. Buddin and E. M. Wakefield.

THE present paper is the outcome of observations and experiments made during the past three years with the object of elucidating, if possible, the life history of the fungus *Rhizoctonia Crocorum* (Pers.) DC. Owing to unexpected difficulties and complications which developed in the course of the cultural work, the results obtained up to the present can only be regarded as preliminary, and are not put forward with any claim to finality. As, however, for various reasons it seems unlikely that any more conclusive results will be obtained by the authors for some time, this account of what has been done is published in the hope that it may be of service to other workers.

The investigation arose out of an identification made in the autumn of 1922. At that time there was received at Kew from the Horticultural Research Station at Long Ashton a stump of black currant (*Ribes nigrum*) which had a dense felted violet growth round the main stem. The growth was sterile, but from previous knowledge of the habit and structure of *Helicobasidium purpureum* (Tul.) Pat. little hesitation was felt in suggesting that it was probably this species. Subsequently, however, it was found that the roots of the *Ribes* showed the bodies known variously as microsclerotia, or better as "infection cushions"

* Paper read at the International Congress of Plant Sciences, Ithaca, N.Y., August 19th, 1926.

or "corps miliaires," which are characteristic of attack by *Rhizoctonia Crocorum*. The question then arose as to whether the first identification had been an error, or whether *Helicobasidium purpureum* could conceivably be the long-sought-for perfect stage of the *Rhizoctonia*.

A priori the latter suggestion seemed to be more feasible than some of those which had been advanced at various times. *Helicobasidium* is a Basidiomycete, to which group the fructification of *Rhizoctonia* would most probably belong. Further, one species, *Helicobasidium Mompa*, is known to be a root-parasite of the mulberry in Japan, and to produce sclerotia on the roots; while another species, *H. longisporum* Wakef., occurred in association with a *Rhizoctonia*-like mycelium on roots of cacao in Uganda, and was suspected of parasitism.

Rhizoctonia Crocorum has long been known in the sterile state as a root parasite of numerous plants, and from time to time various suggestions have been made as to its mode of reproduction. A complete summary of the literature on the subject was given by Duggar (1) in 1915, and his paper should be consulted for further details. The two suggestions which have received most attention are those of Fuckel and Eriksson. The former (2) found a Pyrenomycete, *Leptosphaeria circinans* (Fuck.) Sacc., associated with *Rhizoctonia* on roots of lucerne, and suggested a connection between the two fungi. The same association has also been noticed by other observers. Duggar, however, germinated spores of *Leptosphaeria circinans* and obtained from them a mycelium which had no resemblance to that of *Rhizoctonia*. Eriksson, no doubt influenced by the discovery that the perfect stage of *Rhizoctonia Solani* is a *Corticium* (or *Hypochnus*, as it was first called), examined some material, preserved in spirit thirteen years before, of certain weeds which had been planted in soil inoculated with *Rhizoctonia Crocorum* from carrots. As a result he announced that he had found a *Hypochnus* which he believed to be the perfect stage of the *Rhizoctonia*, and he gave to it the name *Hypochnus violaceus* (3)*. Unfortunately he gave no description whatever of his supposed *Hypochnus*. It is to be noted that he claimed to have found only *basidiospores*, and possibly did not see basidia. In the absence of any confirmatory evidence it has not been possible to accept Eriksson's conclusion.

In a subsequent paper (4) Eriksson used his supposed discovery of a *Hypochnus* stage connected with *Rhizoctonia* on carrot as

* In the 3rd edition, 1926, of Delacroix and Maublanc, *Maladies des Plantes Cultivées*, p. 144, the new combination *Corticium Erikssonii* is used for this supposed perfect stage. As Eriksson's name was a *nomen nudum*, the change is superfluous.

an argument in favour of the view that the Rhizoctonias of carrot and lucerne are distinct, since he had already concluded that the latter was connected with *Leptosphaeria circinans*. He further suggested that the *Rhizoctonia* found on asparagus might be yet a third species and possibly connected with *Diaporthe* (or *Leptosphaeria*) *Asparagi* Fuck. The present authors (5) have already shown that cross-inoculations lend no support to this idea. In 1923 and 1924 they were able to produce typical root-rot in carrots with pure cultures of *Rhizoctonia* isolated from red clover, and since that time they have carried out successful inoculations on various leguminous plants, including lucerne, with strains of *Rhizoctonia* derived from sugar beet, from potato, and from mangold.

Obviously all these speculations as to the connection of associated spore-forms were without experimental proof. This was no doubt partly due to the failure of all the early attempts to grow *Rhizoctonia Crocorum* in pure culture. This difficulty was finally overcome by Van der Lek (6) who, in 1917, succeeded in obtaining pure cultures on malt agar. He did not, however, pursue the work, and his cultures have been allowed to die out.

Not wishing to add yet another unproven guess to an already somewhat formidable list, we determined to test our *Helicobasidium* hypothesis, if possible, by means of pure cultures and inoculations, and to that end set out to obtain material for experiment. Fortunately, just at the time excellent material of *Rhizoctonia* on red clover was received from Mr W. M. Ware, who kindly responded to a request to supply relays of specimens. The successful starting of pure cultures has already been recorded (5). It may be mentioned here that by the methods described in that paper successful isolations of undoubted *Rhizoctonia Crocorum* from various plants have since been made.

Field Observations.

The problem of obtaining fresh material of fertile *Helicobasidium* for the purpose of making cultures for comparison seemed much less likely to be readily solved. The fungus had apparently been found only once in this country, namely at Alresford, Hants, on ash bark. As the species is more often recorded in France, an effort was made to obtain fresh specimens from mycologists there, but without success. Again, however, a fortunate coincidence occurred. Hearing that Mr Ware, who had been continuing his work on the clover disease (7), had found an associated spore form, the authors wrote explaining what they wanted, and inquired whether by chance Mr Ware's fungus might be this species. Mr Ware at once sent drawings and specimens, which the authors were delighted to recognise

as their much wanted *Helicobasidium*. They would like to take this opportunity of expressing their appreciation of the great generosity and helpfulness of both Professor Salmon and Mr Ware, who handed over all their material of the fungus.

(1) On April 30th, 1923, the senior author visited Wye, and in company with Mr Ware examined the field of red clover where both *Rhizoctonia* and *Helicobasidium* had occurred. *Helicobasidium* was found in small areas here and there through the field. It was difficult to see on account of the tall growth made by the clover by this time, but sometimes it was possible to pick out a likely spot by the somewhat sickly yellowish appearance of the plants. The fungus occurred close to the ground, surrounding the bases of the clover stems and the lower leaf-sheaths, and spreading outwards from them to surrounding objects, as oat stubble, grass, stones, soil, etc. Numerous clover plants showing the fungus were dug up and taken away with the surrounding soil intact. The following day the soil was carefully washed away, and in many cases the *Helicobasidium* was found to be associated with plants which showed typical root-rot due to *Rhizoctonia Crocorum* (Plate XIII, fig. 32). The association was so close and the colour of the mycelium of both so similar, that connection seemed most probable, although it was not possible actually to trace the fine hyphal strands continuously from the infection cushions to the fertile *Helicobasidium*. In most of the affected plants the root-rot had proceeded so far that the dead cortex slipped off easily, and frequently only a stump of the tap-root remained. Further, at such a late stage the filamentous mycelium of the *Rhizoctonia* is less evident than in the early stages of disease, and it was sometimes difficult to find any external hyphae running along the diseased roots.

(2) On October 13th, 1924, Miss J. C. Eyre sent a small box of resupinate fungi collected at Ipplepen, near Newton Abbot, Devon. Amongst them was what appeared to be *Helicobasidium purpureum*, though as yet sterile, growing on ash bark and on some small green stems. Miss Eyre fortunately was able to find again the spot whence it came, and in company with her the senior author thoroughly examined the locality about ten days later. The fungus was completely covering a thick ash root which had become exposed just inside a rabbit burrow. Some of the finer roots which were hanging round the mouth of the burrow were also covered with the same beautiful violet felt, while within an area of about a square yard the fungus occurred also on dead twigs and leaves and sometimes attached to the base of the stems of Dog's Mercury (*Mercurialis perennis*), which was practically the sole ground vegetation at that spot.

After a careful preliminary survey it was decided to leave the large mass of mycelium on the ash root undisturbed, in the hope of obtaining spores later. Digging was undertaken round about in order to excavate any other roots that might be harbouring *Rhizoctonia*. Some fine ash roots, an ash seedling, and numerous plants of *Mercurialis* that were in contact with the aerial felt were thus removed and taken away for more detailed examination. After washing to remove the soil, all the fine ash roots, and also the root of the ash seedling, were found to be invested with either fine strands or even fairly thick cords of deep, reddish purple mycelium, running backwards from the aerial felt. In none of the ash roots however were any infection cushions observed.

The *Mercurialis* provided more definite information. Similar strands of the purplish mycelium were found running along the stolons from plant to plant, and in one case were clearly seen to be connected with the purplish felt on an ash twig which had been lying on the surface of the ground beside the plant. Of greatest interest was the fact that this plant, and also many others of *Mercurialis* dug up from the area infested by the fungus, showed dark decayed roots and stolons from which the cortex came away easily. With the aid of a lens, numerous bodies resembling "corps miliaires" were detected on these decayed roots and underground stems, and that this was in reality their nature was confirmed subsequently by microscopic examination. The "corps miliaires" or infection cushions of *Mercurialis* agree exactly in structure with those found on potato, clover, etc., affected with Violet Root Rot, but are slightly larger than those on clover with which they were compared. Plate XIV, figs. 33 and 34, shows a plant of *Mercurialis perennis*, with diseased roots and stolons, the mycelium from which is connected with encrusting mycelium on the adjacent debris. Attempts to isolate the fungus from the roots failed in this case, owing to the fact that they were too badly rotted and consequently contaminated with other more rapidly growing organisms. Fig. 34 shows a typical example of a rotted stolon, with cortex becoming detached, and numerous infection cushions.

Miss Eyre kindly undertook to keep the locality under observation. Investigation of all available records of *Helicobasidium* had indicated that the fructification occurs apparently only in spring. It was hoped therefore that the large mat of mycelium which had been left intact on the ash root would produce spores in the following spring months and thus establish another case of close association of *Helicobasidium* and *Rhizoctonia*. Climatic conditions during the winter were unfavourable, and by April,

1925, only a collapsed brownish membrane was apparent. Fortunately, however, satisfactory evidence has since been obtained. At the beginning of March of the present year (1926) Miss Eyre reported that fresh violet mycelium was appearing again in the same spot, both on the large ash root and on the smaller plants around. Towards the end of March, with the onset of warmer weather, she was successful in finding the fertile *Helicobasidium* hymenium on the aerial felt, and forwarded specimens for confirmation. Examination of these showed that the hymenium had formed sometimes above the old collapsed mycelial weft, and was growing from it, so that there could be no doubt of the continuity of this sporing stage with the mycelium previously observed. In other cases the fructification was not obviously connected with any old mycelial felt.

(3) On April 10th, 1925, Miss Eyre found good fertile specimens of *Helicobasidium* in another locality, and forwarded material for examination. A week later this ground was carefully examined and digging operations undertaken.

Here the *Helicobasidium* occurred on a low hedge-bank which divided a narrow lane from a field. In a small area, a square yard or less, it was found in abundance, encrusting the bases of various plants growing there, notably *Urtica dioica, Digitalis purpurea*, and occasionally small grasses. It occurred also on the bare soil just inside a rabbit burrow*. Examples of all the plants with which the fungus was associated were carefully uprooted and taken away for examination. After the roots had been washed free from soil it was found that those of *Digitalis* and the grasses were perfectly clean and healthy. In the *Urtica*, however, unmistakable root-rot was present, again with infection cushions such as are typical of *Rhizoctonia Crocorum*. Plate XIV, fig. 35, shows an example of *Urtica* with the fructification of *Helicobasidium* at the base of the stem, while the roots and runners bear the infection cushions of *Rhizoctonia*.

The original diagnosis of the fungus on black currant as *Helicobasidium purpureum*, made before its association with *Rhizoctonia* on the roots of the same plant had been observed, has thus been justified. On three further hosts, and in three widely separated localities, there has been found close association of the Basidiomycete with root-rot having the characters of attack by *Rhizoctonia Crocorum*.

* A similar frequent production of the fructification in holes in the ground occurs in the fungus causing Texas root-rot of cotton, *Phymatotrichum omnivorum* (Shear) Duggar. In both fungi the requirements for the production of spores appear to be shade and a humid atmosphere. (See King, C. J., in *Journ. Agr. Res.* XXVI, 1923, p. 407.)

Nomenclature and Description of the fungus *Helicobasidium purpureum*.

The basidiomycetous fungus in question was first described and figured by Tulasne (8, 9) under the name *Hypochnus purpureus*. His description of its habitat, at the foot of small trees or covering living and dead parts of small herbaceous plants, its distinctive violet colour, and his excellent figures of the basidia and spores leave no doubt as to the fungus he had before him.

In 1885 Patouillard (10), having apparently overlooked Tulasne's work, described the species as new and created for it the genus *Helicobasidium*, characterised by the peculiar curved, septate basidia. Fortunately he used the same specific name, *purpureum*, so that no change was necessary when the identity with Tulasne's fungus was recognised. In a later work Patouillard himself cited *H. purpureus* Tul. as a synonym of his *Helicobasidium purpureum* (*Essai Taxon.*, 1900, p. 12). Meanwhile Schroeter (11) had founded the genus *Stypinella* on Tulasne's species; his genus was, however, antedated by Patouillard's and cannot stand.

Helicobasidium purpureum (Tul.) Pat. has been recorded in Europe from several districts of France, from Germany, and at the time the present work was begun once only from England. It has not yet been found in America, but *Helicobasidium Peckii* Burt, founded on an old collection from the Adirondack Mountains, seems to be very closely allied, differing mainly in the colour, in which no trace of violet is mentioned. As the colour of old dried specimens of *H. purpureum* is frequently hardly violet, but cinnamon-drab*, it seems possible that *H. Peckii* may prove to be the same species.

H. purpureum occurs sometimes on bark at the base of trees, on fallen branches, leaf debris, etc., but more often it is found encrusting the bases of small herbaceous plants, after the manner of *Sebacina incrustans* Tul. It is usually found to be quite separable from its above-ground support, and for this reason Bourdot, when describing it in his "Hyménomycètes de France" (*Bull. Soc. Myc. Fr.* xxv, 1909, p. 17), added "il n'est point parasite." It is interesting, however, to note that in the description accompanying Roumeguère's Fungi Gallici Exsiccati, No. 3706 (*Rev. Myc.* 1886, p. 146), evidently compiled from notes made by Barla, it is stated that the fungus sometimes covers a great extent of its support, and envelopes the whole root-system of the plant, which it soon destroys. This

* The colour-terms used throughout this paper are those of Ridgway, *Color Standards and Color Nomenclature*.

particular collection, made by Barla in the neighbourhood of Nice, was described as having a conidial form, which, according to Patouillard (12), occurred in March, before the basidia appeared. The statement is particularly interesting in view of the fact that a conidial fungus, to be described later, has frequently occurred in cultures made by the writers, although so far it has not been seen in nature. The odour of lighting gas, which was attributed to Barla's fungus, has not been noticed in any British specimens of *Helicobasidium*.

As far as available records and the writers' experience show, *H. purpureum* occurs in fertile condition during only a very limited period of the year, roughly from the end of March till the latter part of May, and even then is doubtless dependent on the occurrence of favourable climatic conditions. It is at its best in very mild, moist weather such as occurs in the south of England about April, and it is extremely sensitive to hot sun or drying winds. When growing at the base of closely-set herbaceous plants such as clover, or in a shady wood, it appears to find its ideal conditions.

The fructification consists of an indefinitely effused, fairly thick, dense felt, not at all gelatinous or waxy, of a beautiful purplish or violet colour when at its best. The exact colour varies from light greyish-vinaceous, through livid brown and deep purplish-vinaceous, to dark Corinthian purple. The growing margin is byssoid and paler, while the hymenium is smooth, close but not waxy, and pruinose from the abundant hyaline spores and projecting sterigmata. With age, or in dry weather, the whole fungus becomes much paler in colour, tends to lose the purple tinge, and acquires a cinnamon-drab hue.

The basal hyphae are dark reddish-brown or purplish-brown in colour, septate at rather long intervals, branched, and 5–7 (–10)μ in diameter. Patouillard described occasional clamp-connections at the base of the basidia, but in none of the specimens seen by us has there been any trace of them, though occasional anastomoses of adjacent hyphae occur (Plate XI, fig. 11).

The hymenium consists mainly, if not entirely, of basidia, which are usually to be found in all stages (Plate XI, fig. 1). The young basidium is a cylindrical erect branch arising from one of the subhymenial hyphae. The basidia are hyaline and full of rather densely granular contents, whereby they stand out markedly from the basal and subhymenial hyphae, which tend to become empty at an early stage. The elongating apex of the basidium gradually becomes bent over in a characteristic crozier-like fashion (Plate XI, fig. 1), and soon afterwards transverse septa appear. The sterigmata arise one from each cell of

the basidium, and as a rule two or three only are found, rarely four (Plate XI, figs. 1, 2). They are subulate in form, and vary in length according to the depth of origin, in order to bring the spores above the surface level of the hymenium; the length may be from 10 to 15 or 35μ, and the width at the base 3·5–4μ. The spores are hyaline, ovate, elliptic oblong, or usually somewhat reniform, with practically no apiculus, 10–12 (–15) × 6–7μ (Plate XI, fig. 3).

The cytology of *Helicobasidium* has not been worked out in detail, but such observations on nuclear behaviour as have been made from stained preparations have indicated that the fungus may be interesting in this respect.

The mycelium of *Helicobasidium*, as far as observed in the fructification, is binucleate, the two small nuclei occurring usually rather close together near the middle of each cell (Plate XI, fig. 7, and Plate XII, figs. 13, 14). In the very young basidium there appears to be a single larger fusion nucleus, which remains until the crozier form has been assumed (Plate XII, fig. 15). The stages of division of the fusion nucleus have not been followed, and it is possible that irregularities occur. The cells of the septate basidium, before the development of sterigmata, are uninucleate (Plate XII, fig. 16), but division of this nucleus apparently may occur at a very early stage. Sometimes distinctly one nucleus only has been seen in the sterigma, while the very young spore shows as yet no nucleus (Plate XII, fig. 17). In other cases two nuclei may be observed in the sterigma, or one in the sterigma and one in the young spore (Plate XII, figs. 18–20). The mature spore appears to be usually binucleate (Pl. XII, fig. 21). It is possible however that the nucleus occasionally does not divide before entering the spore, and that a uninucleate spore and mycelium may result.

Morphological comparison of the mycelia of *Helicobasidium purpureum* and of *Rhizoctonia Crocorum*.

At first, before any fresh material was available, a careful examination was made of herbarium specimens of *Helicobasidium* in order to compare more closely the mycelial characters of the fungus with those of *Rhizoctonia*. The hyphae of the two fungi were found to be so much alike that the authors decided that their hypothesis was well worth testing and to this end the cultures of *Rhizoctonia* were begun. Later observations made from fresh specimens of *Helicobasidium* have confirmed the resemblance as to mycelial characters.

The vegetative hyphae of *Helicobasidium* are purple-brown in colour, very even in diameter for long distances, thick-walled and rather rigid when old, and in the septation, mode of

branching, and absence of clamp-connections cannot be distinguished from the typical hyphae of *Rhizoctonia Crocorum* (cf. Plate XI, figs. 11 and 12). Further, short swollen cells such as occur in connection with the sclerotia of *Rhizoctonia*, as figured by Duggar (*l.c.* p. 418), have also been found occasionally in *Helicobasidium*, where the hyphae become closely aggregated. There is also a resemblance between the two fungi in the development of mycelial strands, which adhere closely to the roots or other structures bearing the fungus.

With the object of checking the resemblance in external form, various strains of *Rhizoctonia* and of the growth obtained in culture from spores of *Helicobasidium* have been stained for nuclei. The first strain of *Rhizoctonia* which was isolated, namely that from red clover, was found to have only one nucleus in each cell (Plate XI, fig. 9). On the other hand, strains from potato and sugar beet possess very distinctly two nuclei in each cell, placed usually towards the middle as in the subhymenial hyphae of *Helicobasidium* (Plate XI, fig. 10). There is no difference in the parasitism of these strains, as successful inoculations have been carried out with all three.

Coming now to *Helicobasidium*, growths from spores, though not from single spores, have been stained with the result that a similar variation in nuclear behaviour has been observed. The strains tested were particularly those used in inoculation experiments. Of five of these, three had only one nucleus per cell (Plate XI, fig. 7), one was regularly binucleate (Plate XI, fig. 8), as is the mycelium of *Helicobasidium* in nature, while in the fifth both uninucleate and binucleate cells were present. The significance of these nuclear differences is not at present understood, and the subject would probably repay more exact cytological investigation.

Spore Germination and Growth in Culture of *Helicobasidium purpureum*.

The spores of *H. purpureum* germinate readily in the presence of moisture, and at ordinary room temperatures will put out short germ-tubes within twenty-four hours (Plate XII, fig. 22). In the course of preliminary observations made in hanging drops, it soon became evident, however, that there are difficulties as to further growth. The cytoplasm and nuclei pass into the germ-tube, which continues to elongate very slowly (Plate XII, figs. 22–25). The cytoplasmic contents, however, do not increase in proportion, with the result that there is formed a long empty hypha from which the small amount of apical cytoplasm is cut off by successive transverse walls (Plate XI, fig. 4 and Plate XII, fig. 23). This apparently starved form of

development is particularly marked in sterile water and on Dox's agar, the two media which were used for hanging drops, and most of the spores started on such media sooner or later die out without producing colonies. At the time the cultural experiments with *Helicobasidium* were started the authors had no experience as to suitable media for the growth of *Rhizoctonia*, hence it was necessary to try as many media as possible, both liquid and solid. On all those tried germination was found to be as described, always with the cytoplasm only at the growing apex of an otherwise empty hypha. There were however differences in the degree of further development according to the nature of the medium, and especially according to the relative thickness of sowings of the spores. It was soon noticed that isolated spores were particularly liable to fail (Plate XI, fig. 4), whereas the spores lying close together in a heavy deposit soon began to develop branches from the original germ-tube, and seemed to have better prospects of survival (Plate XI, figs. 5 and 6). For this reason the first stock cultures in 1923 were started from mass deposits of spores, and no attempt was then made to obtain single-spore growths. In some cases small portions of the hymenium were attached to the lids of Petri dishes in such a way that the liberated spores would fall on to the surface of the solid nutrient agar or gelatine. In other cases the spores were collected in moist chambers on clean sterilised glass slips, then suspended in sterile water, and drops of the suspension were spread on the surface of similar solid media. The spore deposits thus obtained were examined microscopically and appeared to be free from any obvious contaminations.

From plates started in this way very slow and apparently pure growth was obtained on several media, most readily on such media as malt extract agar, malt and meat extract agar or gelatine, prune agar, etc. Slight growth occurred on some other media, soil extract agar, Waksman's modified egg albumen agar, etc., but the first mentioned were found to be most suitable and have been used in all subsequent work with spores.

One of the difficulties in dealing with this organism has been its extremely slow rate of growth. In heavy spore deposits, the first visible indication of growth is seen in about four to six days at favourable temperatures, in the form of a slight purplish discolouration of the medium. In about ten days small colonies just visible to the eye are established. With isolated spores growth is much slower. In ten to fourteen days, at room temperatures, such spores may produce only two or three branches from the original germ-tube, while colonies are barely visible to the eye even after a month. If the cultures are incubated at 25° C. growth is a little more rapid. All cultures are charac-

terised by the development of an intense vinaceous colour in the substratum, accompanied usually by the precipitation of small rhomboidal crystals. The aerial mycelium is at first whitish to pinkish lilac. In a few cases it soon assumes more or less of the reddish brown or purplish colour which is characteristic of *Rhizoctonia*, the purple tinge being particularly noticeable on the more acid media such as prune agar. In the majority of cases however the aerial growth remains for a long time pale, and in such cultures there frequently appear in about a month small raised tubercles, which eventually become pustules of conidia of the type which is characteristic of the genus *Tuberculina* (Plate XII, fig. 26).

Most of the known species of *Tuberculina* are found associated with rust fungi, and have been regarded as parasites of the rusts. Hence, when conidia of this type appeared in the first multiple-spore cultures, it was thought probable that they belonged to an intruder, although no trace of such a fungus had been seen in any of the material used for making the spore-deposits. Unfortunately, by the time this difficulty arose it was too late to obtain further fresh material of *Helicobasidium*, and an effort was therefore made to separate what seemed to be two organisms by means of hyphal characters. In some of the cultures there was a radiating marginal zone of darker purplish hyphae, growing closely adpressed to the agar, which were morphologically very like those of *Rhizoctonia*. In other cultures there appeared after a time erumpent, dark purplish, compact masses of hyphae, almost sclerotium-like in appearance (Plate XIV, fig. 36). Such cultures gave the impression of being mixed growths, and it was thought that the darker hyphae must belong to the true *Helicobasidium*. It was not found possible to cut off single hypha tips, but by plating out teased-out portions of the compact masses, growth was obtained in two cases from what appeared to be single hyphal fragments. These two strains (*B* and *C*), when grown further, developed only the dark hyphae, and in fact acquired an appearance similar to that of various strains of *Rhizoctonia*, with no further development of conidia on any medium. They will be mentioned again in connection with inoculation experiments.

At the same time the conidia were plated out, and a few cultures from single conidia were transferred to tubes of malt and meat-extract agar. The further development of these single-spore cultures was surprising, in that they, also, developed at once the characteristic purplish brown mycelium, and remained continuously dark-coloured and quite sterile.

In the spring of 1925 fresh material of *Helicobasidium* was obtained, this time associated with root-rot in *Urtica dioica*.

Further specimens from the same locality were sent in April of 1926. Having now some experience as to suitable media for development of the fungus, the authors started plates in the hope of obtaining growth from single spores. Even on the most favourable media there seems to be always a tendency for many of the more isolated spores to die out after making very little growth. Development from the less isolated spores is more certain, but when several spores have germinated fairly close together, the isolation of guaranteed single-spore growths becomes difficult on account of the curious method of germination already described, with formation of a long (up to 2 mm. or more), wandering, empty tube which becomes almost invisible. Numerous moderately separated spores were transferred to fresh plates as soon as growth seemed promising, and from these a number of practically certain single-spore cultures were obtained.

Cultures on malt agar from single spores have shown variations equally as puzzling as those which originated from mass spore-deposits. The great majority at first produced pale-coloured aerial mycelium, and rather compact, lumpy growth. One or two strains, however, became more or less brownish almost at once, and developed more spreading and closely adpressed hyphae. Many of the former in the 1925 isolations produced conidia, and of the 1926 isolations all the single-spore strains tested produced conidia abundantly when transferred to potato plugs (Plate XIV, fig. 37). Hence there is no doubt that the conidia really belong to the *Helicobasidium* and do not, as was first thought, indicate an impurity. There seems to be an undoubted tendency for the power to produce conidia to be lost after a long period *in vitro*. Cultures which in 1923 were producing *Tuberculina* pustules abundantly now form very few, if any, conidia. Similarly, some of the subcultures from 1925 isolations have now ceased to produce conidia, and one at least has a luxuriant reddish brown growth of aerial hyphae not unlike that of a strain of *Rhizoctonia* from alfalfa.

Description of conidial form, and comparison with other species of *Tuberculina*.

The first indication of the formation of conidia in cultures is the development of small tubercles towards the centre of the colony. Sometimes these occur irregularly, but more often they are arranged in a ring, giving a rosette-like effect. The middle of each tubercle becomes slightly indented and acquires a violet colour and a smooth surface which contrasts sharply with the pale hyphal margin. As the conidia are formed the colour becomes slightly more reddish, the final tint of the conidia in

mass being from vinaceous-fawn to vinaceous-russet. When the conidia are developed abundantly, as on potato plugs, they fall away from the acervuli, and the base and inner walls of the culture tubes become covered with the dusty, vinaceous powder.

The structure of each acervulus is that of the genus *Tuberculina*. In the early stages the conidiophores line the base of a slight depression, but eventually they grow out to form a convex sorus of the usual Tuberculariaceous type. The conidiophores are erect, unbranched, unseptate except at the base, very closely crowded, obclavate, or, after development of the first conidium, slightly pointed at the apex, about 25–35 × 4·5–5·5μ (Plate XII, fig. 26). The conidia are usually globose, 10–16μ in diameter, but may sometimes be elliptical or ovate, 10–18 × 9–15μ (Plate XII, figs. 26, 27). Each conidium has two nuclei, which on germination pass with the cytoplasm into the germ-tube (Plate XII, figs. 28, 30). As in the case of the basidiospores, the cytoplasm follows the growing apex and a long empty tube is left behind (Plate XII, fig. 30). The regular *Tuberculina* pustules just described are typical of comparatively recent isolations. Along with them there occur also conidia borne apparently on ends of ordinary projecting hyphae, and these latter tend to be deeper in colour and more variable in shape. They suggest in fact chlamydospores. In strains which have been growing for a long period *in vitro* the second type of conidium is more abundant (Plate XII, fig. 29).

Comparison with other species of *Tuberculina* leaves no doubt as to the affinities of this conidial form. In habit and in the constant presence of two nuclei the species resembles most closely *T. persicina* Ditm. as described and figured by Cornu (13), Sappin-Trouffy (14) and others. It differs from that species, however, in its larger and sometimes elliptical or ovate spores. The method of germination of the conidia is similar to that described by Cornu (13) for *T. persicina*, and by Tubeuf (15) and Lechmere (16) for *T. maxima*. The latter species also has large conidia, but its mycelium and spores are constantly uninucleate, as observed by Lechmere (16) and verified by us.

With regard to the conidial form already mentioned, which was described by Patouillard as occurring in specimens of *Helicobasidium* from Nice, Patouillard's figure (Tab. Anal. no. 561) represents an arrangement of erect, unseptate conidiophores arising from the coloured basal hyphae, which bears a slight resemblance to the acervulus found in culture. The conidiophores, however, are figured with a much more drawn-out apex, and the conidia are long-elliptic, not at all globose. Unfortunately the specimens of *H. purpureum* var. *Barlae* which are in the Kew Herbarium show no trace of conidia, hence it

has not been possible to check Patouillard's description. None of the material of *Helicobasidium* we have examined has ever shown any development of conidia in nature.

Comparison of the growth in pure culture of *Helicobasidium* and *Rhizoctonia*.

During the period of three and a half years that the cultural work with *Helicobasidium* has been in progress, opportunity has arisen to grow also several strains of *Rhizoctonia* for comparison. In addition to the strain from red clover, whose growth in culture has already been described (5), we have since isolated the fungus from infection cushions formed on potato, sugar beet, mangold, and stinging nettle (*Urtica dioica*)—in the latter case associated with *Helicobasidium*. We have also received from Dr Kotila of Michigan a strain isolated by him from alfalfa, and from the Central Bureau for Cultures at Baarn a strain isolated by Wollenweber from *Beta vulgaris* (variety not stated).

These seven strains, while agreeing in general macroscopic and microscopic characters, such as colour, size and septation of hyphae, etc., yet show considerable variation amongst themselves in cultural characters when grown on the same medium and under the same conditions. Thus on malt extract agar, the medium which has been used constantly in the more recent work, the alfalfa strain, as one extreme type, has always given more luxuriant and more rapid growth than any of the others, and forms a characteristic radiating mycelium which spreads round the sides of the tube. This strain is at first very pale, and always shows a pale marginal growing zone. The potato strain also gives fairly luxuriant aerial growth, but less pale at first, more uniform in colour, and without the striking radiating growth. The strain from red clover is also uniform in colour, and produces less aerial mycelium, most of the growth being closely adpressed to the medium. The strain from *Beta vulgaris* has a general appearance somewhat like the clover strain, but has a tendency to form large, sclerotium-like lumps in old cultures, while that from sugar beet gives also lumpy growth with greater tendency to violet coloration than most forms. The mangold strain comes at the other extreme of the series, the growth being very compact and irregularly lumpy, with little or no spreading mycelium. The fungus isolated from roots of *Urtica* has been, until recently, always paler and slower in growth than any of the others, and has formed scattered lumpy colonies much like the early isolations of *Helicobasidium* from spores. A comparatively recent sub-culture, however, has developed the more normal brownish

spreading mycelium, and all subsequent transfers from this tube have retained this character.

The various strains also vary somewhat when grown on different media, and sometimes at different periods, for no apparent reason. Thus the clover strain on potato plugs sometimes remains dark, but occasionally gives at first a quite pale growth.

The variation in macroscopic appearance in cultures of *Rhizoctonia* is paralleled closely by similar variation observed in the cultures made from spore deposits of *Helicobasidium*. As already noted, the *Rhizoctonia* from *Urtica* resembles most closely the pale-coloured cultures which form the majority in first isolations from spores. The fact that a brown strain has appeared in subcultures from this is exactly similar to the early experience with subcultures from *Helicobasidium*, when growths of such different appearance were obtained that we were for a long time convinced that the original cultures must have been mixed. A further interesting parallel is found in the presence of conidia. When it was noticed that potato plugs favoured the development of conidia, the strains of *Rhizoctonia* were also transferred to that substratum. While most of them gave nothing but the usual sterile growth, the *Urtica* strain developed *Tuberculina* conidia, with typical pustules such as occur in many *Helicobasidium* strains. Further, the mangold strain (which has been proved to cause *Rhizoctonia* root-rot) gave similar conidia, though in this case they were not developed in regular pustules, but resembled rather the conidia of *Helicobasidium* strains that have been grown in artificial culture for a long period (Plate XII, fig. 31).

Another point of resemblance appeared when strain *B* of *Helicobasidium* was grown in Petri dishes and allowed to get rather dry. Here sclerotium-like bodies developed exactly resembling those of the clover *Rhizoctonia* when grown under similar conditions ((5), p. 299).

The temperature relations of the two fungi appear to be alike. The details have not been worked out for *Helicobasidium* as for *Rhizoctonia* (5), but its optimum temperature for growth appears to lie similarly in the neighbourhood of 25° C.

Inoculation Experiments.

Except for the conidia above described as occurring in two cases, no strain of *Rhizoctonia* has yet produced any form of fructification in culture, and there seems to be little hope of obtaining the perfect stage under artificial conditions. Hence the only method of proving a connection between these two

fungi would seem to be that of producing the typical root-rot by inoculation with pure cultures of *Helicobasidium*.

To this end a large number of inoculations of living plants, chiefly various clovers, and occasionally carrot, have been carried out with strains derived from spore cultures of *Helicobasidium*, and at the same time parallel inoculations have been made with the strains of *Rhizoctonia* available. In all cases the plants have been grown in large pots (8″ to 10″). In the first experiments the soil used was the ordinary partially sterilised, potting soil as used at Kew; in later work the pots and soil were sterilised in the autoclave. In working with a fungus such as this, which grows only very slowly in the soil and does not readily produce spores, the danger of accidental contamination is very slight, but as a precaution control pots in adequate numbers have always been used to check results.

The fungus *Rhizoctonia Crocorum* does not appear to be a very virulent parasite under ordinary English conditions. The few inoculations made in open ground have failed, and when diseased clovers have been transplanted to fresh uninfected ground they have recovered and there has been no further spread of the fungus on their roots. Inoculations made in pots have frequently succeeded, but there have also been failures, and the right conditions for infection are not altogether understood. There is some indication that infection takes place more readily in a sandy, well-aerated soil, provided sufficient moisture is present, than in heavy soils. There is also some indication from the results obtained by the authors that after growth for a long period *in vitro* on a medium such as meat-malt-extract agar, on which the fungus makes its most abundant development, the *Rhizoctonia* may lose its virulence. Successful inoculations have, however, been obtained with cultures of both *Helicobasidium* and *Rhizoctonia*.

Of the strains of *Rhizoctonia* isolated from infection cushions on diseased roots, that from red clover has produced root-rot with infection cushions on carrot and on various clovers. The strain from sugar beet has also infected carrot and clovers, including lucerne or alfalfa; while those from potato and mangold have produced root-rot in various clovers. Infection with the clover strain was at first very vigorous, but became less so after the cultures had been maintained for two years. The mangold strain has not given a very high proportion of infections. No result has as yet been obtained from inoculations with the strains isolated by Kotila and Wollenweber, nor with the strain we isolated from roots of *Urtica*.

Successful inoculations with cultures derived from spores of *Helicobasidium* have been less frequent. Three strains, all of

which originated from the first specimens, associated with red clover, have produced the typical symptoms of Violet Root-Rot in clovers and carrot. One result was also obtained early in the work with a fourth strain (*C*), but as the plants used in that experiment had been transplanted from a field, thought to be free from infection but not certainly so, there is some doubt as to whether the infection was due to the inoculation. No other inoculations with *C* have given positive results. In 1925 a very large number of pots were inoculated with strains of both multiple and single-spore origin, derived from the *Helicobasidium* associated with *Urtica*, but none of these strains has so far given any infection. It is just possible that the *Urtica* strain may not be infective to the host plants used in these experiments, but the matter will be discussed further in the next section.

The history of the strains of *Helicobasidium* which have given positive results is as follows:

A. Originated from a spore deposit made in April 1923, which was transferred directly to a tube of malt agar on April 19th. This culture produced the compact purple growth described above, portions of which were teased out and plated. Promising-looking hyphae from these growths were transferred to malt agar on June 18th, 1923. A tube used for inoculation on March 24th, 1924, gave no result, but a malt-meat extract subculture from it made on the same day gave positive results when used on April 15th, 1924.

B. The hymenium used for making the original spore deposit on Dox's agar on April 10th, 1923, was growing on oat stubble which was intermixed with affected red clover.

Transfers were made as follows: April 16th, potato agar; May 12th, prune agar. This tube developed the purple compact growths described, fragments of which were plated out on June 3rd (malt agar). A growth which appeared to be coming from a single hyphal fragment was transferred to a tube of malt-meat-extract agar on June 28th, 1923. Subcultures from this tube were those afterwards used for inoculations.

C. The early history of this strain is the same as that of *B*. From the plate made on June 3rd a growth which had certainly originated from a small fragment of a single hypha was transferred to a tube of Jardox gelatine on July 2nd, 1923. Subcultures from this were those subsequently used for inoculations and called *C*.

F. A spore deposit was made on April 14th, 1923, and transferred to spinach agar. Subcultures from this to malt agar were made on May 4th and on June 14th. The cultures had remained reddish brown from the beginning and had produced

no conidia, hence no plating out had been considered necessary. The tube of June 14th and subsequent subcultures were used in inoculation experiments.

The table on p. 135 sets out the method of treatment and results in the successful experiments made with these strains.

Discussion of Results.

The important fact which emerges from the work just recorded is that there is a very intimate association between the Basidiomycete *Helicobasidium purpureum* and the root parasite known as *Rhizoctonia Crocorum*, whatever the nature of the relationship may be. Root-rot, characterised by the presence of the "corps miliaires" or infection cushions which are typical of *Rhizoctonia Crocorum*, has been found in every locality investigated where *Helicobasidium* has been found. Further, the association is so close that in two strains of *Rhizoctonia* isolated in the usual way from infection cushions, conidia similar to those obtained from *Helicobasidium* have been produced in cultures.

The supposition that *Helicobasidium* represents the perfect stage of *R. Crocorum* is supported by these facts of intimate association, by the morphological characters of the hyphae of the two fungi, by their similar rates of growth and temperature relations, and by the results of inoculation of living roots by certain strains derived from spore-cultures of *Helicobasidium*.

The last point is the most important evidence in favour of direct genetic connection between *Helicobasidium* and *Rhizoctonia*. The experiments were carried out with careful controls, and except in the first series of pots the soil used was thoroughly sterilised in the autoclave. These precautions, and the fact that it has been always the same strains, *A*, *B*, and *F*, which have given infections, though with some evidence of declining virulence in the course of time, seem to preclude the possibility of accidental infection. It is true that the results are open to the objection that the strains used were derived from original mass spore deposits, and not from single spores; but care was taken to observe that the spores shed were free from any obvious contaminations, and repeated examination of the specimens of *Helicobasidium* used has revealed no other spore-form than the basidiospores of this fungus.

At the same time, the rather widely different appearance of many of the primary spore isolations of *Helicobasidium* from most of the vegetative isolations of *Rhizoctonia*, and the variable behaviour of cultures, does seem to indicate a possibility that *Helicobasidium* may be another species living in close association with *Rhizoctonia*, so close that separation by

Table showing details of successful inoculations with strains of Helicobasidium.

	Plant	Treatment	Date of Inoculation	Strain	Result	Remarks
1	Red clover	Soil from Kew stock partially sterilised, seed sown 5. VII. 23, transplanted when plants had several leaves.	3. IX. 23, 2. XI. 23	*B*	+ (11. IV. 24)	4 good infections with infection cushions, 2 doubtful, out of total 10
2	Carrot	Soil from Kew stock partially sterilised, seed sown 5. VII. 23, transplanted when plants had several leaves	21. IX. 23, 2. XI. 23	*B*	+ (16. III. 24)	4 plants with infection cushions out of 10
3	Carrot	Same soil as in 1, not sterilised	Not reinoculated	*B*	+ (10. IX. 24)	1 plant badly rotted, 16 clean
4	Mixed clovers	Pots autoclaved, soil inoculated when seed sown	15. IV. 24	*F*	+ (17. IX. 24)	Infection on 3 plants out of 6
5	Alsike	Pots autoclaved, seedlings transplanted from old control pot of 1924	28. VI. 25	*F*	+ (13. X. 25)	5 plants out of 9 infected; soil rather sandy
6	Carrot	Pot autoclaved, seed sown 17. IV. 25 in old control pot of 1924, transplanted 14. V. 25	28. VI. 25	*B*	+ (13. X. 25)	1 plant infected out of 5
7	Mixed clovers	Plants transplanted from field	14. IV. 24	*C*	+ (18. XI. 25)	Few plants infected 1925; doubtful case, long period, and original soil not sterilised
8	Alsike	Soil autoclaved, seeds sown in autoclaved soil and seedlings transplanted to 2 small pots	28. VI. 25	*A*	?+ (Oct. 1925)	No infection cushions formed, but one plant showed slight weft of hyphae on root
9	Mixed clovers	Soil autoclaved, inoculated when seed sown	15. IV. 24	*A*	+ (5. I. 25)	50 % of numerous plants in pot infected; tap-root destroyed in some instances

ordinary cultural methods is difficult or impossible. It has been already described how the appearance of *Tuberculina* conidia in their first isolations led the authors to suppose that these were mixed growths. There is no doubt that this conviction caused progress to be slower than it might otherwise have been. With the successful development of single-spore growths, the conidial form has been shown to belong to *Helicobasidium*. There still remains the difficulty, however, that five out of the seven strains of *Rhizoctonia* studied have never produced conidia, and that successful inoculations with *Helicobasidium* have been much less frequent than those with *Rhizoctonia*.

If infection could be obtained with a single-spore culture the case would be proven. Unfortunately the only single-spore cultures we have at present are those derived from the fungus associated with *Urtica*, and as already noted neither the *Rhizoctonia* nor any of the spore strains isolated from this host has as yet given any infection. It is possible that this particular strain is not pathogenic to the plants used in our experiments. On the other hand, such cross-inoculations as have been carried out with strains of *Rhizoctonia* from different host-plants have tended to disprove the existence of any great biologic specialisation in this fungus.

It is obvious that one of the most pressing needs is for more exact information as to the conditions under which *Rhizoctonia* will infect living roots. There have been many failures even in experiments with known infective strains; hence negative results have not the same importance they would have in the case of a more virulent parasite.

Another, as yet unexplained, complication is the extraordinary variation in the appearance of cultures from time to time. There are two main types of growth; one is pale-coloured, compact and lumpy, and this usually produces conidia on suitable media; the other consists of spreading purplish brown hyphae which remain dark-coloured and never produce conidia, but may sometimes form sclerotium-like bodies. When subcultures are made from the dark mycelium they retain these characters and never again produce the pale form of growth, a phenomenon which is illustrated by the history of strains *A*, *B*, and *C* of *Helicobasidium* and by the recent appearance of a dark-coloured, apparently permanently sterile strain in a subculture from the conidia-producing *Rhizoctonia* from *Urtica*.

It is tempting to suppose that *Helicobasidium* is an extremely plastic organism, from which mutants may readily arise. Some support for such a view is provided by the fact that in Petri-dish cultures, both of *Helicobasidium* and of *Rhizoctonia*, a curious sectoring effect is sometimes observed. Sectors of an otherwise

pale-coloured colony may develop the deeper brownish colour; or the hyphae of one sector may suddenly begin to grow much more rapidly than the rest of the colony, so that a markedly lobed outline is produced in place of the previous regular circular shape.

If the explanation of the growth differences observed is to be found in such a tendency to variation (or perhaps mutation), it is conceivable that the pathogenicity of the fungus is subject to similar variation. If that is so, the non-success of so many inoculations made with spore cultures may be due to the fact that the majority of strains are but feebly if at all parasitic, whereas occasionally a more virulent form appears which will produce *Rhizoctonia* root-rot on a suitable host plant. The fact mentioned above, that the mangold strain of *Rhizoctonia* (which produces conidia) is rather less virulently parasitic than a quite sterile form like that from clover, agrees with the results of experiments with *Helicobasidium*, where conidia-bearing strains have produced no infection, and all successful inoculations have been achieved with the derived sterile strains.

From the taxonomic point of view a further interesting question is opened up by the discovery that *Helicobasidium* possesses a conidial stage which morphologically and cytologically is indistinguishable from certain species of the genus *Tuberculina*. The known species of *Tuberculina* are usually found in association with rust fungi, on which they have been supposed to be parasitic, and they have not hitherto been genetically connected with any higher form. In this connection there has arisen an interesting coincidence, which may, or may not, be of significance. In searching through the literature the authors discovered the description of *Rhizoctonia Menthae* B. and Br. (17), in which mention is made of globose conidia. Berkeley and Broome referred the fungus to the genus *Rhizoctonia* on account of the presence of sclerotia, and a weft of violet hyphae resembling those of *R. violacea* (*R. Crocorum*). On examination of the type specimen of *R. Menthae* in the British Museum, it was found that the conidia described are those of a *Tuberculina*, which has practically replaced the accidia of *Puccinia Menthae*, only a few aecidiospores remaining to prove the existence of the usual association.

Summary.

1. Fertile *Helicobasidium purpureum* has been found in very close association with root-rot characterised by infection cushions of *Rhizoctonia Crocorum* in distinct localities, on (*a*) red clover, (*b*) *Mercurialis perennis*, (*c*) *Urtica dioica*.

2. In order to test the possible connection of the two fungi, they have been studied morphologically, culturally, and as to pathogenicity.

3. Morphologically the hyphae of *Helicobasidium* are exactly similar to those of *Rhizoctonia*, having the same type of branching, septation, and absence of clamp connections. They also resemble one another in nuclear characters, both fungi having sometimes one but more often two nuclei per cell.

4. Seven strains of *Rhizoctonia Crocorum* have been compared with numerous spore isolations of *Helicobasidium* as to cultural characters. Both fungi show considerable variation in colour and type of growth. Conidia belonging to the genus *Tuberculina* are frequently produced in cultures of *Helicobasidium*, and similar conidia have been found in strains of *Rhizoctonia* isolated from *Urtica* and from mangold. In cultures of *Helicobasidium* which originated from multiple spores there sometimes develop in subcultures strains which acquire the dark colour characteristic of the sterile *Rhizoctonia* strains, and which remain henceforth sterile. A similar variation has arisen in the *Rhizoctonia* isolated from *Urtica*, which at first produced only pale growth, with a tendency to form conidia.

5. Successful inoculations, with the production of typical root-rot, have been obtained with four strains of *Rhizoctonia*, and with three of the dark-coloured strains derived from *Helicobasidium* spore cultures. The host plants used were various legumes and carrot. In every case precautions were taken to exclude the possibility of accidental infection, and adequate controls were used.

6. The strains of *Helicobasidium* which have proved infective all originated from specimens associated with red clover. None of the strains from *Urtica*, nor the *Rhizoctonia* isolated from *Urtica*, has up to the present produced any infection on clovers or on carrot. Other strains of *Rhizoctonia*, however, have not given evidence of any specialisation in parasitism.

7. The bearing of the observations made is discussed. While there have been inconsistencies in behaviour, it is possible that these are due to the fact that the organism is very variable, and that not all the strains are equally parasitic. It has also to be borne in mind that practically nothing is known as to the conditions for infection with *Rhizoctonia Crocorum*.

The balance of the evidence is considered to favour the view that *Helicobasidium purpureum* (Tul.) Pat. is the perfect stage of *Rhizoctonia Crocorum* (Pers.) DC.

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DESCRIPTION OF PLATES

Plate XI.

Fig. 1. *Helicobasidium purpureum*. Vertical section of sporophore showing hymenium and part of subhymenial tissue. × 450.

Fig. 2. *H. purpureum*. Two basidia. × 500.

Fig. 3. *H. purpureum*. Spores. × 500.

Fig. 4. *H. purpureum*. Germination of an isolated spore, showing poor growth and gradual dying of hyphae, with possible origin of two colonies. The dotted portions contain protoplasm, the remaining hyphae being empty.

Figs. 5 and 6. *H. purpureum*. Late germination of spores in a mass deposit on meat-malt-extract agar, showing favourable influence of the presence of other colonies. × about 450.

Fig. 7. *H. purpureum*. Hyphae from strain *F* in pure culture, showing two nuclei in each cell. × 500.

Fig. 8. *H. purpureum*. Hypha from strain *C* in pure culture, showing one nucleus per cell. × 500.

Fig. 9. *Rhizoctonia Crocorum*. Hypha from red clover strain in pure culture, showing one nucleus per cell. × 500.

Fig. 10. *R. Crocorum*. Hypha from sugar beet strain in pure culture, showing two nuclei in each cell. × 500.

Fig. 11. *H. purpureum*. Hyphae on stem of *Urtica dioica*. Drawn from specimen in Jaap, Fung. Sel. Exs. No. 389. × 500.

Fig. 12. *R. Crocorum*. Surface hyphae from potato. × 500.

PLATE XII.

Figs. 13–20. Successive stages in the development of basidium and spores. × 800. Fig. 13. Terminal cell of hypha with two nuclei. Fig. 14. Beginning of fusion. Fig. 15. Young basidium with large fusion nucleus. Fig. 16. Basidium after completion of divisions of fusion nucleus. Fig. 17. Development of sterigmata and spores. Fig. 18. Division of nucleus after entering sterigma. Fig. 19. Passage of first nucleus into spore. Fig. 20. Second nucleus entering spore.

Fig. 21. *H. purpureum*. Binucleate basidiospores. × 800.

Fig. 22. *H. purpureum*. Germination of basidiospores. × 800.

Fig. 23. *H. purpureum*. Germinating basidiospore, showing passage of cytoplasm to extreme tip of germ tube. × 800.

Figs. 24, 25. *H. purpureum*. Development of first branches of germ tube from basidiospore, showing binucleate condition. In Fig. 25 the nuclei have divided but a wall has not yet been formed. × 800.

Fig. 26. *H. purpureum*. *Tuberculina* conidial form as developed in pure culture, strain from red clover: (*a*) portion of conidial layer of pustule, (*b*) isolated conidiophores. × 500.

Fig. 27. *H. purpureum*. Conidia from pure culture, *Urtica* strain. × 500.

Fig. 28. *H. purpureum*. Conidia, showing two nuclei. × 800.

Fig. 29. *H. purpureum*. Conidia formed in old cultures, not in definite pustules (? chlamydospores). *Urtica* strain. × 500.

Fig. 30. *H. purpureum*. Germination of *Tuberculina* conidia. × about 350.

Fig. 31. *R. Crocorum*. Conidia developed in culture of mangold strain on potato plug. × 500.

PLATE XIII.

Fig. 32. *Red clover*. Showing fructification of *Helicobasidium purpureum* on petioles and stems at ground level, and infection cushions of *Rhizoctonia Crocorum* on the tap-root. Natural size.

PLATE XIV.

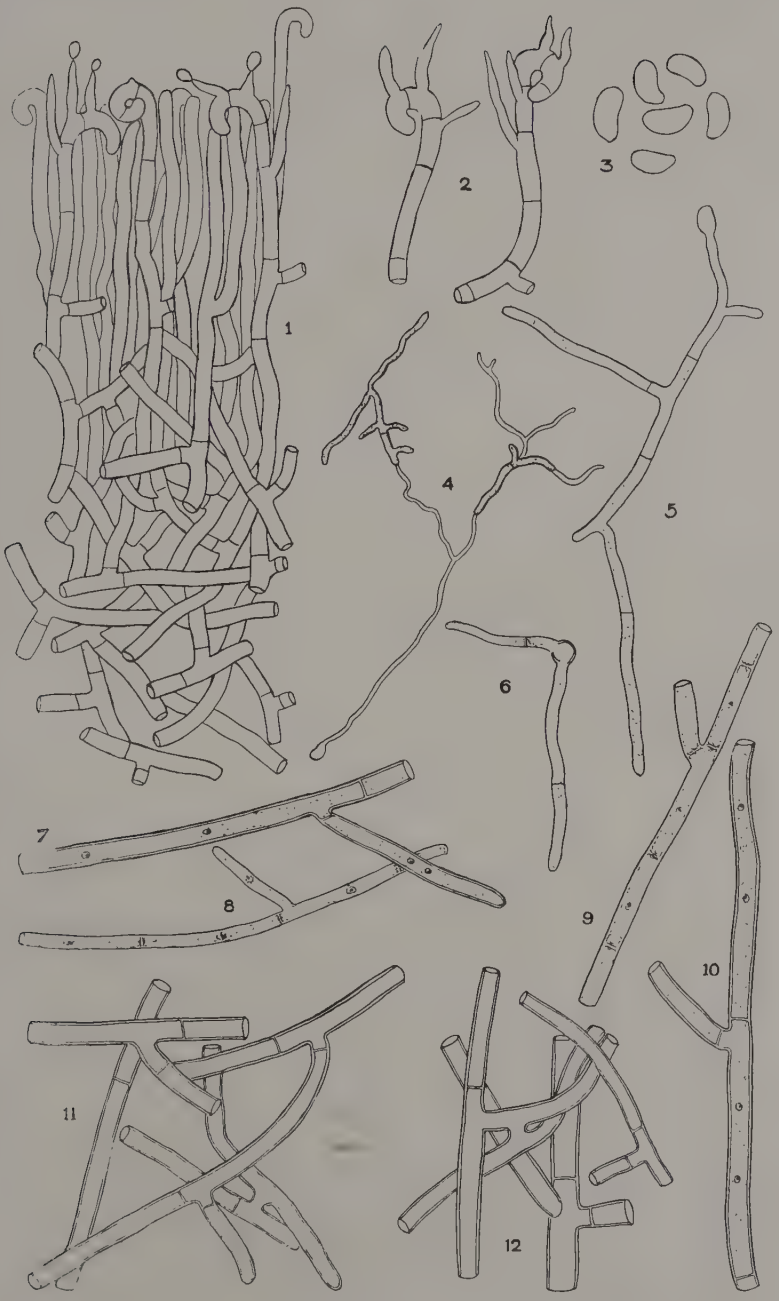
Fig. 33. *Mercurialis perennis*. Fallen ash twig on left covered with superficial felted mycelium; small root in centre showing numerous infection cushions of *R. Crocorum*.

Fig. 34. Portion of an underground runner of *Mercurialis perennis* showing rotted cortex with infection cushions of *R. Crocorum*.

Fig. 35. *Urtica dioica*. Showing fructification of *Helicobasidium purpureum* on stem above ground, and infection cushions of *R. Crocorum* on roots and underground runners.

Fig. 36. *Helicobasidium purpureum* (red clover strain). First isolations from spores, showing two kinds of growth.

Fig. 37. *Helicobasidium purpureum* (*Urtica* strain). Single-spore culture on potato plug, showing numerous pustules of conidia of the *Tuberculina* type.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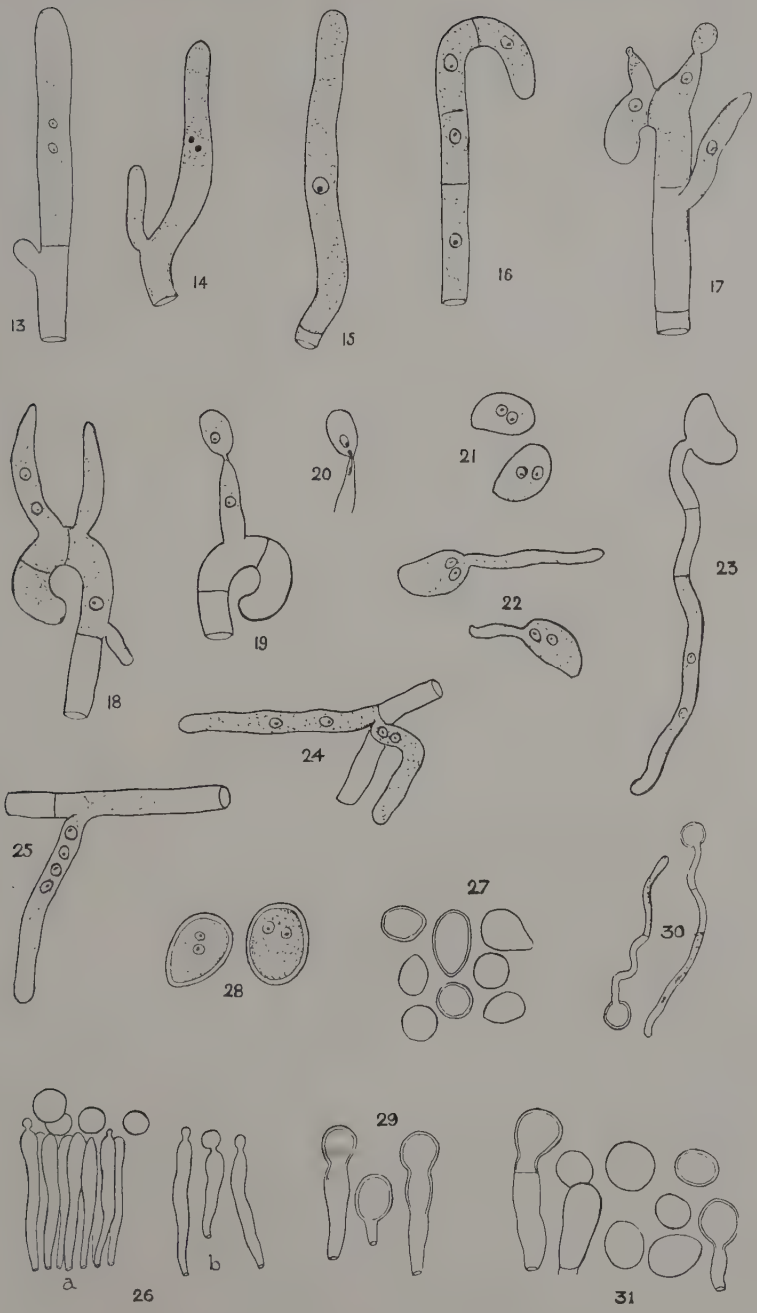
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